The integral role that Notch proteins play in the normal development and tissue homeostasis of metazoan animals is orchestrated through the tightly regulated proteolytic processing of its Negative Regulatory Region (NRR). The NRR is composed of three linear notch repeats (LNR) followed by a heterodimerization (HD) domain that is N-terminal to the transmembrane region. Proteolytic Notch processing begins during transport to the cell surface with the cleavage of the HD domain at site S1 by a furin-like protease so as to produce a non-covalent heterodimer of one extracellular (NEC) and one transmembrane (NTM) subunit. Ligand binding at NEC initiates Notch activation by facilitating ADAM-type-metalloprotease-dependent extracellular cleavage at an NTM site (S2) that enables a subsequent intramembrane γ-secretase-mediated cleavage at site S3. These cleavages permit the translocation of the intracellular domain of Notch from the cell membrane to the nucleus to activate transcription. In this work we present the algorithms we have developed to evaluate the relative importance of specific amino acids for the structural stability and functionality of the NRR. These include predictive models derived from bioinformatic analysis such as phylogenetic inference and computational protein modeling as well as experimental data from multiple biophysical and biochemical techniques such as differential scanning calorimetry (DSC), circular dichroism (CD), and pull-down assays. Our results provide valuable insight to the mechanism of action of Notch.

3026-Pos Board B73

Macromolecular Crowding Affects Stability Properties: A Comparison Study For Titin And Ubiquitin

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Macromolecules can occupy a large fraction of the volume of the cell and this affects many properties of the proteins inside the cell, such as thermal stability and rates of folding. We present a comparison of the effects of molecular crowding in ubiquitin and titin. We have used an atomic force microscope based single molecule method to measure the effects of macromolecular crowding on the mechanical stability of these proteins. We used dextran as the crowding agent with two different molecular weights, with concentrations varying from zero to 300 grams per liter in the buffer solution. The results show that the forces that are required to unfold molecules are enhanced when high concentration of dextran molecules is added to the buffer solution.

3027-Pos Board B74

Properties Of Contact Matrices Induced By Pairwise Interactions In Proteins

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The total conformational energy is assumed to consist of pairwise interaction energies between atoms or residues, each of which is expressed as a product of a conformation-dependent function (an element of a contact matrix, *C* matrix) and a sequence-dependent energy parameter (an element of a contact energy matrix, *E* matrix). Such pairwise interactions in proteins force native *C* matrices to be in a relationship as if the interactions are a Go-like potential [N. Go, Annu. Rev. Biophys. Bioeng. **12**:183, 1983] for the native *C* matrix, because the lowest bound of the total energy function is equal to the total energy of the native conformation interacting in a Go-like pairwise potential. This relationship between *C* and *E* matrices corresponds to (a) a parallel relationship between the eigenvectors of the *C* and *E* matrices and a linear relationship between their eigenvalues, and (b) a parallel relationship between a contact number vector and the principal eigenvectors of the *C* and *E* matrices; the *E* matrix is expanded in a series of eigenspaces with an additional constant term, which corresponds to a threshold of contact energy that approximately separates native contacts from non-native ones. These relationships are confirmed in 182 representatives from each family of the SCOP database by examining inner products between the principal eigenvector of the *C* matrix, that of the *E* matrix evaluated with a statistical contact potential, and a contact number vector. In addition, the spectral representation of *C* and *E* matrices reveals that pairwise residue-residue interactions, which depends only on the types of interacting amino acids but not on other residues in a protein, are insufficient and other interactions including residue connectivities and steric hindrance are needed to make native structures unique lowest-energy conformations. Reference: Phys.Rev.E, **77:**051910, 2008.

3028-Pos Board B75

The Charge on Beta-Lactoglobulin A and B under Self-associating Conditions

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From electrophoretic and hydrodynamic measurements, the charge on monomeric proteins may be calculated accurately. It is not clear, though, how to analyze data from a self associating system. β-Lactoglobulin (β-LG) is a major whey protein abundant in cow milk. It has several genetic variants, is stable and self-associates under well characterized conditions. These factors make β-LG a good model to be used in the study of net charge and self-association. Both β-LG A and β-LG B were analyzed at pH 3, in a range of salt concentrations and protein concentrations. Under these conditions, self association is favored by higher salt concentrations. The mobility of β-LG was determined using capillary electrophoresis, with the Stokes radius determined using analytical ultracentrifugation. Self-association of β-LG A was characterized using sedimentation equilibrium. For both β-LG A and β-LG B, as ionic strength increased the mobility of the faster-moving peak decreased. With increased concentrations of β-LG A and β-LG B, the apparent mobility of this peak stayed the same. However, the boundary shapes observed in capillary electrophoresis were consistent with β-LG undergoing a mass-action association. It is difficult to interpret the capillary electrophoresis data quantitatively. Accordingly, membrane confined electrophoresis experiments are being used to determine the charge change on association.

3029-Pos Board B76

Volume changes in protein folding: A Modular Approach

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Pressure effects of protein conformational transitions, which are manifest via the associated changes in molar volume, remain rather poorly understood, and regrettably so given that they are clearly linked to changes in the magnitude and type of hydration that accompany such transitions. Given that the role of solvent in protein energetics and structural dynamics remains one of the key questions in the field of protein folding, a better understanding of the physical basis for the volume changes that accompany folding reactions could provide direct means to quantify this differential solvation. We have recently begun high pressure studies on the folding of the ankyrin repeat domain of the protein Notch bearing 7 ankyrin repeats (Nank7) and a series of smaller constructs differing is the number of repeats, and/or their sequences. We reasoned that such a system would provide a means of incrementally testing the role of the size of the hydrophobic core, and the importance of the specific amino acid sequence in determining the volume change upon unfolding, ΔVu. We present here a complete P-T equilibrium and p-jump kinetic study of the full-length Nank7 construct, as well as on a number of the smaller constructs. We find that the volume change of the full-length construct depends strongly upon temperature, and that the expansivity of the transition state ensemble is similar to that of the unfolded state, while its molar volume is closer to that of the folded state. Preliminary results on two very small constructs indicate that the total volume change is a linear combination of the volume change associated with the unfolding of each individual repeat.

3030-Pos Board B77

Effect of Macromolecular Crowding on Protein Folding Stability

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The interior of a cell contains ~300 g/l macromolecules. In order to understand the biophysical properties of proteins under in vivo conditions, it is necessary to consider the crowding effects of bystander macromolecules [1]. Statistical-thermodynamic models show that crowding increases the chemical potentials of both the folded and the unfolded states of a protein and predict a modest increase in folding stability [2], which has been experimentally confirmed in our group [3]. Here we report additional experimental results for crowding effects on FKBP12 mutant. At a fixed concentration measured in weight per volume, we observed an optimal dextran size at which stability increase is maximal, just as predicted previously [4]. In addition, we found that the stabilizing effect of a mixture of dextran 6K and Ficoll 70 is greater than the sum of the two individual crowding agents. These findings will have profound implications for understanding macromolecular crowding inside cells.

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